

ASPECTS OF THE DETERMINATION OF EQUILIBRATION RATES BY NMR SPECTROSCOPY¹

John D. Roberts

Crellin Laboratory, California Institute of Technology, Pasadena, California
91125, USA^a

Abstract - The origins of the line-shape method for determining the rates of chemical and conformational equilibration by NMR spectroscopy are reviewed for the purpose of illustrating the factors which should be considered in obtaining accurate rate data by this very useful technique. Besides a number of illustrations of how changes in rates of conformational equilibration change NMR spectra taken from the literature, some new results using a simple and direct procedure for measurement of NH exchange rates of amides and hydrazines by natural-abundance ¹⁵N NMR will be presented.

INTRODUCTION

Without question, nuclear magnetic resonance (NMR) spectroscopy has greatly deepened our knowledge of chemical compounds and their reactions. No small part of this has been the application of NMR to the study of rate processes, either directly by following the rate of change of signal intensities with time or indirectly of equilibration rates by analysis of line shapes. The latter procedure will be the subject of this paper.

DEVELOPMENT OF THE LINE-SHAPE PROCEDURE FOR MEASURING EQUILIBRATION RATES BY NMR

In this era of hectic change in both science and our daily lives, there is now a trend (or fad) for establishing the basis for how we got to where we are. "Searching for our roots" is the popular phrase. The taproot of NMR is of course the demonstration by Purcell² and Bloch³ in 1946 that NMR signals could be obtained of protons in liquids and solids. This work of Bloch and Purcell was done with magnets of low homogeneity and the discovery of the chemical shift in 1950,⁴ and of spin-spin splitting in 1951⁵ required substantially greater resolution.

The first rootlet hinting at exchange processes was the discovery in 1951,⁶ that the chemical shift of the OH proton of ethanol showed a temperature dependence, and this was almost immediately interpreted correctly by Liddel and Ramsey⁷ as arising from changes in association equilibria with temperature. "Since this association is by a hydrogen bond involving the hydrogen in the OH group, this hydrogen should experience a different magnetic shielding in the associated and unassociated state. If the correlation time for the lifetimes of the different states is sufficiently small (less than a millisecond), the hydrogen resonance will be observed at the frequency corresponding to the average shielding for the states." This rootlet was greatly nourished by the work of Gutowsky and Saika⁸ who showed that separate OH resonances for water and ethanoic acid could not be observed in mixtures of these substances but instead there was a common OH resonance whose position was a linear function of the mole fractions of the components. Although Gutowsky and Saika⁸ did not provide any experimental examples of fast, intermediate, and slow exchange, they did use the Bloch equations to show theoretically how the line shapes would change with different preexchange lifetimes.

Apparently, the first published report of averaging of both shifts and spin-spin couplings came from Weinberg and Zimmerman⁹ who showed that when water was added to ethanol, separate OH resonances could be obtained until the concentration of water reached about

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20% by weight. Above that concentration a single average line was obtained. Changes in the spin-spin coupling pattern of the CH_2 were also observed over the concentration range where the lines coalesced. Curiously, there is no reference to Gutowsky and Saika or to Weinberg and Zimmerman in a later, otherwise superb, paper in 1956 by Arnold¹⁰ who constructed a permanent magnet, remarkable for the time, with which proton spectra could be taken at 30 MHz having a resolution of about 0.5 Hz. This magnet permitted Arnold to discover the second-order splitting between the CH_2 and CH_3 groups of ethanol, as well as produce excellent spectra of the collapse of the OH triplet of non-exchanging ethanol when made more than $5 \times 10^{-6}\text{M}$ in HCl. Arnold calculated line shapes for the OH proton of ethanol as a function of the exchange rate, τ , and showed how these could be correlated with the observed line shapes. The difficulty of getting reproducible line shapes as a function of HCl concentration prevented this paper from being the definitive initial example of the power of the NMR method for measuring equilibration rates.

In 1955, Phillips¹¹ demonstrated that rotation about the C-N bond of *N,N*-dimethylmethanamide was slow and in 1956 Gutowsky and Holm¹² showed how the gradual averaging of the chemical shifts of the methyl groups with increasing temperature could be used to obtain an activation energy for the bond rotation. This could have been the definitive initial paper on determination of rates from line shapes but, in fact, it gave NMR measurements of rates a very bad aroma for several years. The problem was that Gutowsky and Holm related the rates to line separations and got an activation energy for rotation of *N,N*-dimethylmethanamide of 7 ± 3 kcal which for a process for which having ΔG^\ddagger of about 19 kcal means that ΔS^\ddagger has to be -32 eu which seems quite unreasonably large. Subsequent measurements of activation energies of rotation about amide bonds by many researchers gave a wide disparity of values ranging up to 25 kcal. Two difficulties were common. One was that temperatures of NMR samples are not easy to control precisely or easy to measure. The other was the Gutowsky-Holm treatment, which requires substantial correction whenever the chemical-shift difference changes with temperature. If the chemical-shift difference decreases with temperature, as it does with *N,N*-dimethylmethanamide, before chemical-shift averaging really becomes important, and line separations are used as the criterion of averaging, the rate will appear to change over a wider temperature range than it should. This means that the activation energy will appear to be smaller than its actual value. The theoretical spectra shown in Figure 1, calculated by Gutowsky and Holm's equation show

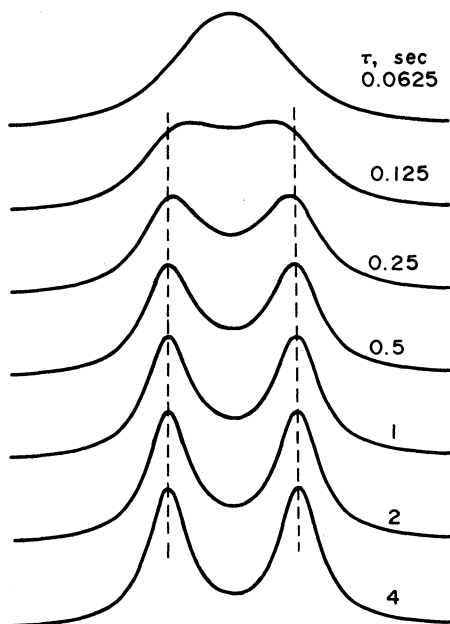


Figure 1. Line shapes calculated as a function of τ for two-site exchange by equations of Gutowsky and Holm¹² with $6\omega = 6$ Hz, $T_2 = 0.9$ sec, showing how there is little movement of the peaks toward one another until near the coalescence point.

how the line separations do not in fact change very much with increasing rate until the sample gets close to the coalescence point. The coalescence point is of course easy to measure and, as a result, there is usually good agreement between different investigators as to the free energy of activation--whether or not they agree on the enthalpy of activation by a factor of two.

The enthalpy of activation can be determined reasonably accurately by measuring the coalescence temperature for the same sample in spectrometers with different magnetic fields and thus a range of chemical-shift differences. The relative rate at the coalescence point is proportional to the chemical shift so that between 30 MHz and 600 MHz one could cover a rate range of a factor of 20 rather accurately.

Subsequent to the work on rotational barriers of amides came the discovery that barriers to rotation could be determined for C-C single bonds^{13, 14} and of ring inversion in cyclohexane.¹⁵ The floodgates for study of conformational equilibration were then open and, because of the complexity of the spectra which began to be observed, better procedures for calculating line-shape changes as a function of rate became necessary.

Before proceeding to these, it may be well to consider why the signals are averaged and it is interesting that both the Bloch equations which are regarded as essentially classical equations and the uncertainty principle predict that line broadening, coalescence, and then line narrowing will occur as the exchange rate increases. The uncertainty principle suggests that, if the lifetime of a state is short, there will be an uncertainty in the energy of the state and line broadening will occur. Line narrowing above the coalescence point is a consequence of the fact that the uncertainty in the energy of the averaged state becomes less. The Bloch equations predict line broadening by inclusion of the T_1 and T_2 relaxation times as well as the exchange rates and these rates essentially amount to uncertainty in the lifetimes of the states.

A more precise quantum mechanical approach to the problem of calculating line shapes was introduced by Sack¹⁶ and Kaplan¹⁷ using the density-matrix procedure. This was a great advance, because it permitted introduction of spin-spin coupling for situations in which the chemical shifts and couplings are of comparable magnitude (AB vs. AX in NMR parlance). The Kaplan procedure was soon programmed to calculate line shapes for AB-type systems¹⁸ and many new interesting applications became available. Still later Binsch¹⁹ used the Liouville representations to produce much more efficient algorithms for computing line shapes and these can be used with very complex spin systems, which as we will see offer substantial advantage for measuring larger changes in rate for a particular variety of exchange.

The line-shape procedure as described here is only one of several methods for determining rates of equilibration by NMR. Pulse methods such as the spin-echo technique²⁰ are very powerful as is also double resonance.²¹ Because the interpretation of spectra produced by these procedures is complex, they have not been used much by organic chemists and consequently they will not be discussed further here.

One might well wonder whether equilibration rates for a reaction $A \rightleftharpoons B$ measured by such an esoteric procedure as looking at line broadening in NMR spectra are really the same rates which one might determine by optical rotation, titration and the like, or even by following the change of signal intensity in an NMR tube when pure A goes to equilibrium with B. This point has been investigated and the differences in rate are not larger than the experimental precision of the measurements.²²

SOME PRACTICAL EXAMPLES OF THE LINE-SHAPE PROCEDURE

In many cases, the line-shape method is too difficult or impossible to apply because the NMR spectra are just too complicated to be calculated even without the extra complications produced by exchange. An example is cyclohexane below the temperature where the signals of the axial and equatorial hydrogens are averaged to a single line. One technique which has been used with great success by Anet²³ is to use massive deuteration so that there is only a single proton left in the molecule. The line-shape problem for cyclohexane- d_{11} (with deuterium decoupling) then reduces to the one presented by a simple two-site, no spin-spin coupling system discussed earlier. Another approach is to use fluorine-labeling²⁴ because fluorine has large chemical-shift differences and useful information can be obtained over

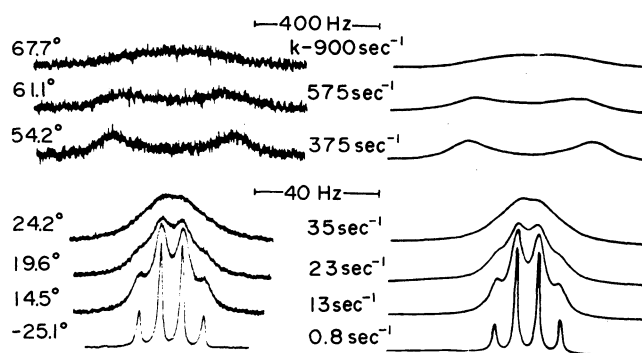
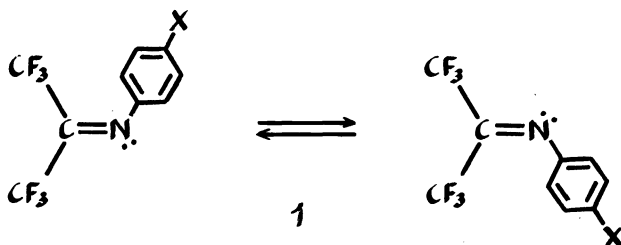


Figure 2. Comparison of experimental ^{19}F spectra of 1 as a function of temperature, below and at the main coalescence point, with calculated spectra as a function of $k (=1/\tau)$ in sec^{-1} , $X = -\text{F}$.

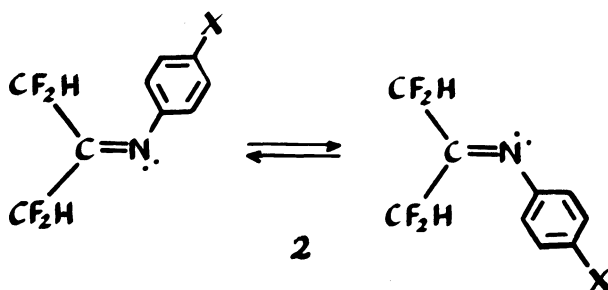
both a wider temperature range and at lower temperatures by observing fluorine than is possible by observing protons. The problem with fluorine labeling is that if one is really interested in activation parameters for the unlabeled compound, it is hard to be sure that the fluorines are not themselves causing unusual effects.²⁵

In general, massive deuteration is not desirable if it can be avoided. The reason is that spectral complexities are extremely useful in extending the range of temperatures over which rates can be measured by the NMR procedure. An excellent example is provided by the (1-(trifluoromethyl)-2, 2, 2-trifluoroethylidene)-4-substituted benzenamines (1) which undergo rather rapid degenerate geometrical isomerization at room temperature (Figure 2).²⁶



The chemical-shift differences between the fluorines of the trifluoromethyl groups of 1 when isomerization is slow are large and there is, in addition, the expected quartets from long-range spin splittings involving the non-equivalent fluorines. These couplings are washed out over about a 40° change in temperature and an additional 40° is required to reach coalescence. Measurements of the rate above the coalescence point can only be made by measuring the line width of the single averaged fluorine resonance of the trifluoromethyl groups, and while this is reasonably accurate it does not compare with fitting the subtle line-shape changes of the quartet in the slower exchange region.

A current study²⁷ of the corresponding difluoromethyl derivatives 2 shows that these substances have substantial NMR advantages over 1, because of the extra complications introduced in the spectra by the ^1H - ^{19}F spin-spin splittings (Figure 3). Comparison of Figures



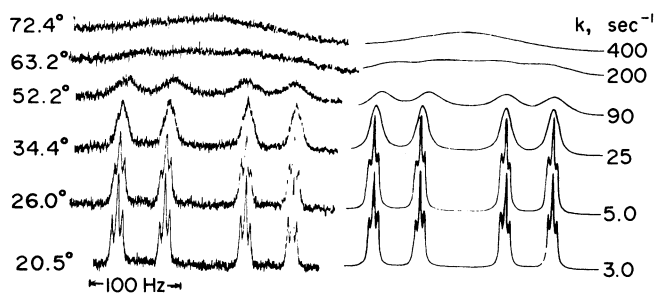
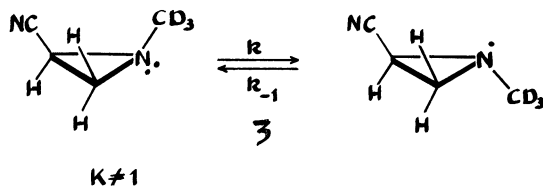


Figure 3. Comparison of experimental ^{19}F spectra of 2 as a function of temperature, below and at the main coalescence point, with calculated spectra as a function of k ($= 1/\tau$) in sec^{-1} , $X = -\text{NO}_2$.

2 and 3 shows that, when isomerization is slow, the ^{19}F spectrum of 2 is a quartet of triplets and we can follow the collapse of first the triplets and then the quartets to the coalescence point. Above the coalescence point (Figure 4), the spectrum gradually splits into a doublet of triplets as expected for an $A_2A_2'XX'$ arrangement in the fast-exchange limit. This greatly extends the range over which the rate can be measured reasonably accurately. The total range encompassed by the changes in line shape covers a range of rates in the order of about 25,000 which is orders of magnitude larger than possible by measuring coalescence points at different magnetic fields or line separations.

The epitome of line-shape analysis has been recently provided by Binsch²⁸ for the $ABC \rightleftharpoons DEF$ system of the ring protons of *N*-trideuteriomethyl-2-cyanoazacyclopropane (3) which undergoes both slow to fast (nondegenerate) inversion at nitrogen and a change of the equilibrium constant K with increasing temperature. The extraordinary changes of the proton spectrum with temperature and the way that these can be duplicated by theoretical calculations are a joy to behold.



Natural-abundance ^{13}C NMR spectra have been widely used for the study of equilibration rates since the first determination of activation parameters for inversion of 1, 1, 3, 3-tetramethylcyclohexane in 1970.²⁹ An excellent review on this subject was published in 1977 which gives activation parameters for more than 180 different compounds.³⁰ With most organic compounds, proton-coupled ^{13}C spectra will be too complex to be useful. Because of the low natural-abundance of ^{13}C , proton-decoupled ^{13}C spectra of exchanging

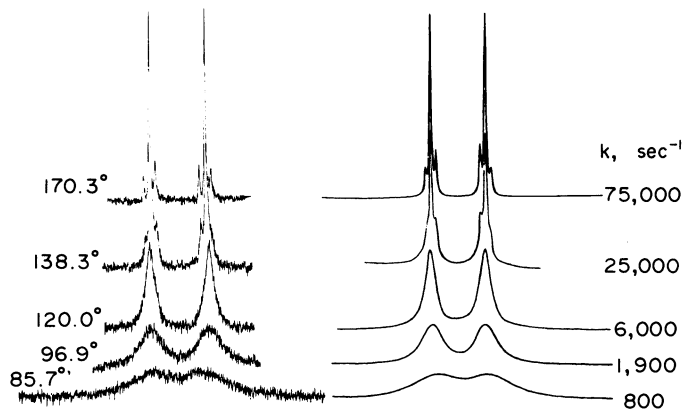
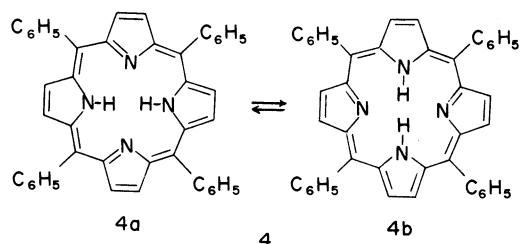


Figure 4. Same as Figure 3 but above the coalescence point.

systems only involve averaging of chemical shifts unless some magnetic nucleus such as fluorine or phosphorus is present in the molecule. Proton-decoupled ^{13}C spectra lack the fine structure which helps in extracting rates from changes in line shapes as discussed above and, if the chemical shifts are large, it may be difficult to have a large enough signal-to-noise ratio in the coalescence region to obtain accurate rate data. Supplementation of the line-shape procedure by the double-resonance spin-saturation procedure²¹ appears to be very useful with ^{13}C spectra.³⁰

Nitrogen-15 NMR has so far had relatively limited use in rate studies. At the natural-abundance level, the sensitivity of detection is about 6×10^{11} more difficult than protons at the same equivalent concentration and this means that either rather large samples or enrichment in the isotope are necessary. Serious problems with ^{15}N NMR are the great variability of the relaxation times of the nucleus, the rather extreme sensitivity of the resonances of many kinds of nitrogen to paramagnetic ions, and the variability of the nuclear Overhauser enhancement (negative) produced by proton decoupling.

An illustrative example of ^{15}N chemical-shift averaging has been published for meso-tetraphenylporphyrin **4**.³¹ A number of attempts to obtain the natural-abundance ^{15}N



spectrum of this substance were unsuccessful under conditions which were known to be satisfactory for related structures. The compound was then prepared enriched to the 95% level in ^{15}N and, when the spectrum of the product was taken, it was found to give a single resonance with a line width of about 180 Hz at 18.25 MHz. This suggested an exchange process such as an equilibrium between 4a and 4b and, when the sample was cooled, the broad singlet became a doublet with a chemical-shift difference of 109 ppm (Figure 5). The coalescence temperature for **4** of about 15°C suggests that ΔG^\ddagger is about 11 kcal in reasonable agreement with ΔG^\ddagger obtained from the changes of the proton spectrum of **4** with temperature.³²

Similar exchange broadening occurs at room temperature when **4** is dissolved in 2-propanone containing less than the equivalent of trifluoroacetic acid to form its dication **5**. No ^{15}N resonances were detected at room temperature because of being close to the coalescence point for equilibration of **4a** with **4b** and both with **5**.

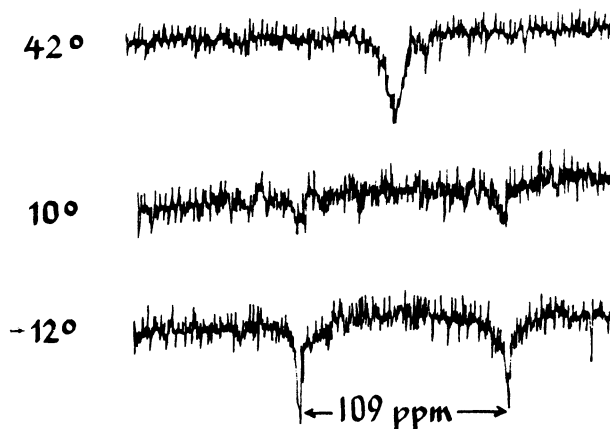
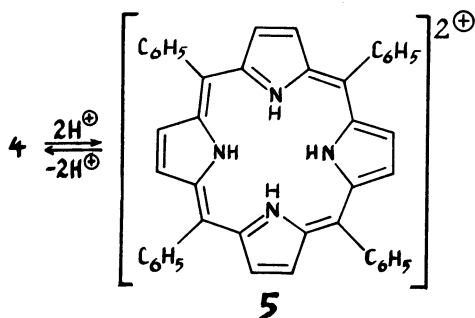


Figure 5. Changes in the proton-decoupled ^{15}N spectrum of **4** at 18.25 MHz with temperature in trichloromethane solution.



The ^{15}N spectra of Figure 6 show that, at -10°C , the equilibrium between 4 and 5 is slow and 5 gives a sharp narrow line because all of its nitrogens are equivalent. The lines of 4 are broad because the $4a \rightleftharpoons 4b$ equilibration is not completely frozen out at -10° . At 38° , $4a \rightleftharpoons 4b$ is above the coalescence temperature for the nitrogen resonances of 4 and exchange with 5 produces substantial line broadening. This is an interesting set of rates because exchange between the imidazole tautomers and between imidazole and imidazolium ion is very fast under comparable conditions.³³

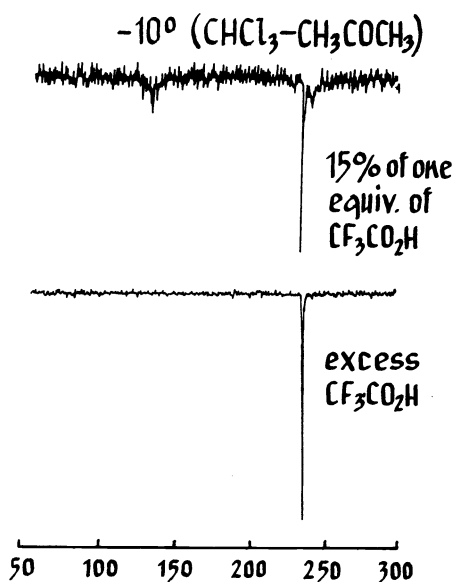


Figure 6. The ^{15}N spectra of 4 in a 2-propanone - trichloromethane mixture at -10°C containing trifluoroethanoic acid.

Nitrogen-15 NMR has recently shown to be very useful at the natural-abundance level to measure acid- or base-catalyzed competitive exchange proton rates of hydrazines, guanidines and amides. Exchange between the hydrogens of phenyldiazane is one example.³⁴ The gated proton-coupled ^{15}N spectra of neat phenyldiazane in Figure 7 show ^{15}N -H couplings that indicate at 20°C exchange of the $-\text{NH}$ -proton must be quite slow because of the sharpness of the N1 doublet. On the other hand, exchange of the $-\text{NH}_2$ protons is fast enough to produce significant line broadening of the N2 triplet resonance. At 60°C , slight exchange broadening of the N1 doublet is evident but the triplet resonance of N1 has degenerated to a broad singlet. The pattern of these exchange rates is expected on the basis of the low basicity of N1 compared to N2. Surprisingly, the exchange rates of the protons attached to N1 and N2 are roughly the same in methylsulfinylmethane, ethanol and ethanol-water (Figure 8). Less surprising is the increase in exchange rate in the more acidic solvents trifluoroethanol and trifluoroethanoic acid.

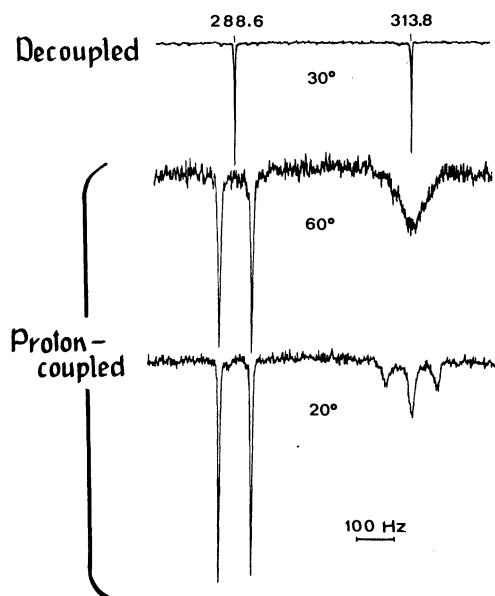
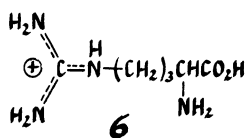


Figure 7. Natural-abundance ^{15}N spectrum of neat phenyldiazane as a function of temperature and proton decoupling. The doublet on the left in the proton-coupled spectrum arises from the $-\text{NH}-$ nitrogen.

Recently, *ab initio* calculations have been reported³⁵ which suggest that the NH_2 nitrogens of the carbamimidoylamine group of the conjugate acid of arginine (6) should be substantially more positive than the NH nitrogen.



This would suggest that the protons on the $-\text{NH}_2$ groups should undergo base-catalyzed exchange much faster than that on the $-\text{NH}-$ group. The changes in the proton-coupled ^{15}N spectra of 6 in water as a function of pH (Figure 9) indicate that this expectation is not realized. The doublet of the $-\text{NH}-$ nitrogen and the triplet of the $-\text{NH}_2$ nitrogens broaden almost equally rapidly.³⁶ Thus, if one allows for a statistical factor of two favoring $-\text{NH}_2$ over $-\text{NH}$, it is clear that the $-\text{NH}-$ protons exchange more rapidly than the $-\text{NH}_2$ protons.

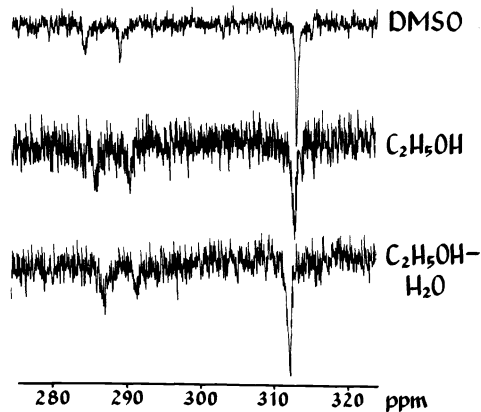


Figure 8. Natural-abundance ^{15}N spectra of 20% v/v phenyldiazane in various solvents at 30°C , protons coupled.



Figure 9. Natural-abundance ^{15}N spectra of 1.5 M arginine in water as a function of pH, protons coupled.

This same technique has been used to study competitive exchange rates of NH protons of ureas and thioureas,³⁷ lactams^{38, 39} and thiolactams,³⁹ and amides.^{37, 40} The exchange rates of the NH_2 protons of primary amides are hard to measure with proton spectra because of ^{14}N quadrupole relaxation, which usually occurs at just the right rate to greatly broaden the NH proton resonances. The same thing occurs with secondary amides, such as RCONHCH_3 , but with these the spin-spin splitting of $-\text{CH}_3$ proton resonances by the NH proton can be used as a probe of exchange rates.⁴¹

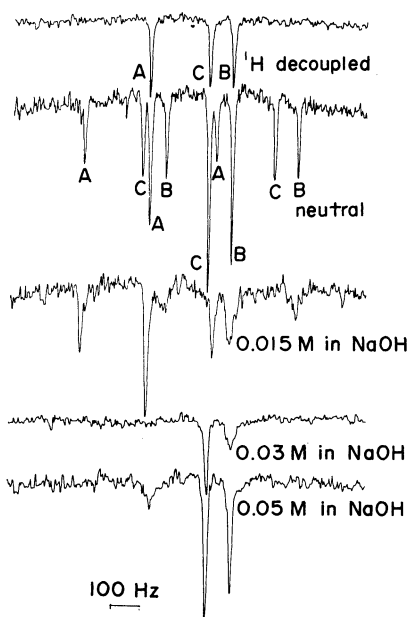


Figure 10. Natural-abundance ^{15}N spectra of a mixture of butanamide (A), benzenecarboxamide (B), and nicotinamide (C) in methylsulfinylmethane, either neutral or with added sodium hydroxide.

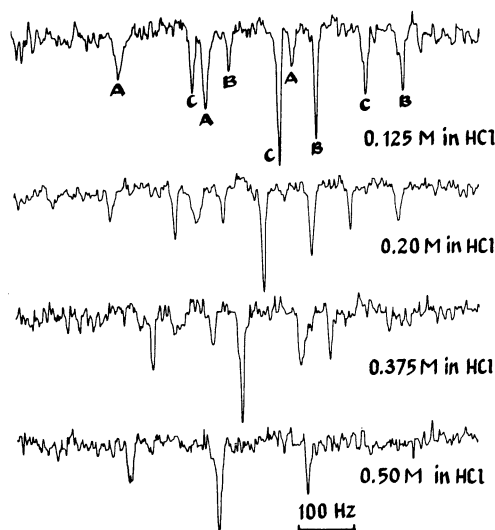


Figure 11. Same as Figure 10 except that hydrochloric acid added rather than sodium hydroxide.

The power of ^{15}N NMR to measure relative exchange rates for primary amides is illustrated by Figures 10 and 11 which show spectra of solutions containing butanamide, benzenecarboxamide and 3-azabenzene-carboxamide (nicotinamide) in methylsulfonamide solution as a function of different amounts of added acid and base.⁴⁰ With added base, the rate ratios are approximately 3-azabenzene-carboxamide:benzenecarboxamide:butanamide as 100:10:1 while, in acid, the ratio is about 1:1000:5000. In acid solution, the ring nitrogen of the 3-azacarboxamide becomes protonated and makes the $-\text{NH}_2$ nitrogen less able to accept a proton than the other amides. In basic solution, the superior electron-attracting powers of the 3-azaphenyl and phenyl groups assist the removal of the $-\text{NH}_2$ protons.

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